PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Applicant's or Agent's file reference 340647/17975	**FOR FURTHER ACTION**	See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)
International application No. PCT/FR00/00622	International filing date *(day/month/year)* 15/03/2000	Priority date *(day/month/year)* 15/03/1999
International Patent Classification (IPC) or national classification and IPC A61K35/74		
Applicant PIERRE FABRE MEDICAMENT et al.		

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 10 sheets including this title page.

   ☒ This report is also accompanied by ANNEXES, i.e. sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Instruction 607 of Administrative Instructions of the PCT).

   These annexes consist of a total of 5 sheets.

3. This report contains indications relating to the following items:

   - I ☒ Basis of the report
   - II ☐ Priority
   - III ☐ Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
   - IV ☐ Lack of unity of invention
   - V ☒ Reasoned statement according to Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
   - VI ☒ Certain documents cited
   - VII ☒ Certain defects in the international application
   - VIII ☒ Certain observations on the international application

Date of submission of the demand 09/10/2000	Date of completion of this report 31.05.2001
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INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No. PCT/FR00/00622

I. Basis of the report

1. This report has been drawn up on the basis of the following elements *(the replacement sheets received by the receiving office in response to an invitation according to Article 14 are considered in the present report as "originally filed" and are not annexed to the report as they contain no amendments (Rules 70.16 and 70.17).):*

Description, pages:

1-17 as originally filed

Claims, No.:

1-33 received on with the fax of 16/03/2001

Drawings, sheets:

1/2,2/2 as originally filed

2. With regard to the **language**, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.

These elements were available or furnished to this Authority in the following language which is:

☐ the language of a translation furnished for the purposes of international search (under Rule 23.1(b)).

☐ the language of publication of the international application (under Rule 48.3(b)).

☐ the language of the translation furnished for the purposes of international preliminary examination (under Rule 55.2 and/or 55.3).

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international preliminary examination was carried out on the basis of the sequence listing:

☐ contained in the international application in written form.

☐ filed together with the international application in computer readable form.

☐ furnished subsequently to this Authority in written form.

☐ furnished subsequently to this Authority in computer readable form.

☐ The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.

☐ The statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished.

4. The amendments have resulted in the cancellation of:

   ☐ the description, pages

   ☐ the claims, Nos.

   ☐ the drawings, sheets/fig

5. ☐ This report has been written disregarding (some of) the amendments, which were considered as going beyond the description of the invention, as filed, as is indicated below (Rule 70.2(c)):

   (All replacement sheets comprising amendments of this nature should be indicated in point 1 and attached to this report).

6. Additional observations, if necessary:

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty	Yes:	Claims	3, 4, 13, 14, 17, 18, 20, 21, 29-33
	No:	Claims	1, 5-12, 15-16, 19, 22-28
Inventive Step	Yes:	Claims	-
	No:	Claims	1-33
Industrial Applicability	Yes:	Claims	1-33
	No:	Claims	

2. Citations and explanations

see separate sheet

VI. Certain documents cited

1. Certain published documents (Rule 70.10)

   and/or

2. Non-written disclosures (Rule 70.9)

see separate sheet

VII. Certain defects in the international application

The following defects in the form or contents of the international application have been noted:

see separate sheet

VIII. Certain observations in the international application

The following observations on the clarity of the claims, descriptions, and drawings or on the question whether the claims are fully supported by the description, are made:

see separate sheet

As regards point VIII

Observations relating to the international application (clarity)

1. Claim 1 lacks clarity within the meaning of Article 6 of PCT because it defines the use of the membrane fraction of Klebsiella pneumoniae/antigen (or hapten) combination in terms of mechanism of action ("intended to orient the immune response toward a Th1 type and/or mixed Th1/Th2 type response directed against said antigen or hapten, in which response the Th1 response is close to or greater than the Th2 response") and of desired result. Indeed, the mode of action by which the membrane fraction (coupled to an antigen) acts as effective adjuvant against the agents from which the antigens are derived is not considered as a therapeutic indication.

   The selection of a Th1 response in relation to a Th2 response undoubtedly constitutes an immunological effect, but it cannot, on its own, be considered as a therapeutic application. The discovery on which the invention is based certainly constitutes an important contribution from the scientific point of view, but it nevertheless requires the existence of a practical application, in the form of a real defined treatment of any pathological condition for it to be considered to provide a contribution of a technical order in relation to the state of the art and that it is an invention which can be protected by a patent.

   Precisely to this effect, the description lists examples of such pathological conditions, namely infectious diseases and cancer (see page 1, lines

9-13), which should be able to be treated according to the present invention.
However, because of the functional definition given of the subject matter claimed, the scope of claim 1 is not limited to the treatment of said pathological conditions, but covers, on the contrary, an indeterminate number of other pathological conditions.
Conversely, the use of the Klebsiella pneumoniae membrane fraction combined with an antigen or a hapten should relate to a therapeutic application (for example according to claim 22).

As regards point V

Reasoned statement according to Rule 66.2(a)(ii) as to novelty, the inventive step and the possibility of industrial application; citations and explanations in support of this statement

1. Reference is made to the following documents:

D1: FR-A-2 766 192 (PF MEDICAMENT) 22 January 1999 (1999-01-22)

D2: FR-A-2 718 452 (PF MEDICAMENT) 13 October 1995 (1995-10-13)

D3: FR-A-2 726 472 (PF MEDICAMENT) 10 May 1996 (1996-05-10)

D4: FR-A-2 748 476 (PF MEDICAMENT) 10 May 1996 (1997-11-14)

D5: FR-A-2 471 785 (PF MEDICAMENT) 21 December 1979 (1979-12-21)

Document D5 was not cited in the international search report.

D6: FR-A-2 596 064 (PF MEDICAMENT) 25 September 1987 (1987-09-25)

2. The passage, page 3, line 29 - page 4, line 2, implies that the P40 protein is included in the definition of the Klebsiella pneumoniae membrane fraction.

The result is that the subject matter of claims 1, 5-12, 15-16, 19, 22-29 is not considered as novel within the meaning of Article 33(2) PCT since the prior art documents reveal that the protein OmpA P40 derived from membranes of bacteria of the genus Klebsiella (see in particular D1, page 2, lines 7 - page 5, line 17; see also D2, claims 7-10; D3, page 1, line 25-31 and D4, page 8, line 12 - page 9, line 18) constitutes an adjuvant agent. Thus, this protein in itself constitutes a membrane fraction, which is also envisaged in the present application (see page 6, lines 28-30). Furthermore, this protein or fragment derived therefrom, in association with an epitope of an infectious agent (for example peptides derived from the respiratory syncytial virus G protein as in D1-D3), is used as a vaccine, or against tumor cells.
The various elements stated in claims 5-9, 11-12, 22-28 are also present in documents D1-D3.

3. It appears that the subject matter of claims 1-33 does not involve an inventive step within the meaning of Article 33(3) PCT.

3.1 It does not appear clearly which technical problem claim 2 has to solve, and it therefore does not appear to provide an inventive element.

3.2 Claims 3 and 4 are directed at the use of membrane fractions obtained according to two different methods.

It appears that the definition of the method of obtaining membrane fractions to be used according to the invention made them novel since it is clear that the methods described in claims 3 and 4 do not lead to the production of protein P40, but to fragments of bacterial membrane, which then clearly distinguishes the subject matter of the claims.

However, the method of claim 3 is a common method for isolating membranes which is used in the laboratory (centrifugation, heating, treatment with a proteolytic enzyme, then washes and finally sonication). This method is also described in application FR 2 471 785 (D5) for the extraction of ribosomes (see page 2, line 19 - page 4, line 7).

The method of claim 4, for its part, is already described in D6, application FR 2 596 064 (example 1).

Consequently, these methods do not confer an inventive step since persons skilled in the art, knowing them well from D5 and D6 (which apply to the same bacterium) would then have used them to obtain membrane fractions containing the P40 protein, as a simpler alternative for production compared with the

adjuvant composed of the purified protein alone.

3.3 Claims 13, 14, 16-18, 20-21 are not revealed in the prior art and are therefore novel, but are not considered as being inventive.
Claims 13 and 14 relate to a method of coupling with a bifunctional reagent, which constitutes a common alternative, and does not therefore exhibit an inventive character.
Likewise, the agents which carry said membrane fraction or which regulate the immune response according to claims 16-18 and 20-21 respectively represent completely obvious solutions with the aim of a better stability and of enhancing the immunogenecity of the vaccines of the invention.

4. Claims 29-33:

Claim 29 contains a contradiction since it defines the method according to the claim for a second therapeutic application. Should these methods be explicitly mentioned, this contradiction would disappear.
In addition, a pharmaceutical composition containing a membrane fraction of this type is novel in relation to the purified P40 protein, but does not involve an inventive step according to the same reasoning as for claims 3 and 4 (see paragraph 3.2).

As regards point VI

Some documents cited

Some published documents (Rule 70.10)

Application No. Patent No.	Date of publication (day/month/year)	Date of filing (day/month/year)	Priority date (validly claimed) (day/month/year)
WO 00/27432	18.05.2000	08.11.1999	06.11.1998

This document relates to the use of a protein OmpA in combination with a hapten or an antigen in order to target the denditric cells (APC) for the treatment of various diseases.
This disclosure appears to be detrimental to the novelty of claims 1, 5-18, 22-23 in regional phase, at the EPO.

Application No. Patent No.	Date of publication (day/month/year)	Date of filing (day/month/year)	Priority date (validly claimed) (day/month/year)
WO 00/48628	24.08.2000	17.02.2000	17.02.1999

This document discloses the use of Klebsiella OmpA associated with an antigen or a hapten for treating in particular various forms of cancer, in a similar manner to the present application. This document appears to be relevant as to the novelty of claims 1, 5-18, 22-23 in regional phase, at the EPO.

Application No. Patent No.	Date of publication (day/month/year)	Date of filing (day/month/year)	Priority date (validly claimed) (day/month/year)
WO 00/48629	24.08.2000	17.02.2000	17.02.1999

This document discloses the use of Klebsiella OmpA associated with a peptide for treating in particular melanomas. This document appears to be relevant as to the novelty of claims 1, 5-18, 22-23 in regional phase, at the EPO.

Application No. Patent No.	Date of publication (day/month/year)	Date of filing (day/month/year)	Priority date (validly claimed) (day/month/year)
WO 00/50071	31.08.2000	24.02.2000	24.02.1999

This document discloses the use of Klebsiella OmpA associated with HCG in particular for treating cancer or fertility. This document appears to be relevant as to the novelty of claims 1, 5-18, 22-23 in regional phase, at the EPO.

As regards point VII

Deficiencies in the international application

1. The passage of the description, page 12, lines 6-7 should not appear since it clearly indicates that the methods for isolating membrane fractions seek protection, whereas this does not appear at all in the present claims.

2. Contrary to what is required by Rule 5.1 a) ii) PCT, the description does not indicate the relevant prior state of the art disclosed in documents D1-D4 and does not cite these documents.

CLAIMS

1. The use of a Klebsiella *pneumoniae* membrane fraction combined with an antigen or hapten for the preparation of a pharmaceutical composition intended to orient the immune response toward a Th1 type and/or mixed Th1/Th2 type response directed against said antigen or hapten.

2. The use as claimed in claim 1, characterized in that the membrane fraction comprises at least membrane fractions of two different bacterial strains.

3. The use as claimed in either of claims 1 and 2, characterized in that the membrane fraction is prepared by a method comprising the following steps:

a) culture of said bacteria in a culture medium allowing their growth followed by centrifugation of said culture;

b) where appropriate, deactivation of the lytic enzymes of the bacterial pellet obtained in step a), followed by centrifugation of the suspension obtained;

c) extraction and removal of nonmembrane proteins and of nucleic acids from the pellet obtained in step a) or b) by at least one cycle of washing the pellet in an extraction solution;

d) digestion of the membrane pellet obtained in step c) in the presence of protease enzymes, followed by centrifugation;

e) at least one cycle of washing of the pellet obtained in step d) in physiological saline and/or in distilled water; and

f) ultrasonication of the pellet obtained in step e).

4. The use as claimed in either of claims 1 and 2, characterized in that the membrane fraction is prepared by a method comprising the following steps:

a) culture of said bacteria in a culture medium allowing their growth, followed, where appropriate, by centrifugation;

b) freezing of the culture medium or of the pellet obtained in step a) followed by thawing and drying of the cells;

c) removal, by means of a DNase, of the nucleic acids from the dry cells obtained in step b) which have been resuspended;

d) grinding of the cells obtained in step c) and clarification of the suspension obtained;

e) precipitation, in an acid medium, of the suspension obtained in step d) and removal of the pellet;

f) neutralization of the supernatant obtained in step e) containing the membrane suspension, followed by dialysis and concentration of the membrane suspension; and

g) sterilization of the concentrated membrane suspension obtained in step f).

5. The use as claimed in one of claims 1 to 4, characterized in that said antigen or hapten is chosen from the antigens or haptens specific to an infectious agent or from the antigens associated with tumor cells.

6. The use as claimed in claim 5, characterized in that said antigen or hapten is chosen from peptides, lipopeptides, polysaccharides, oligosaccharides, nucleic acids, lipids or any compound capable of specifically directing the Th1 type and/or mixed Th1/Th2 type immune response against an antigen or hapten specific to an

infectious agent or an antigen associated with a tumor cell.

7. The use as claimed in one of claims 1 to 6, characterized in that said antigen or hapten is coupled or mixed with said membrane fraction.

8. The use as claimed in one of claims 1 to 7, characterized in that said antigen or hapten is covalently coupled with a supporting peptide to form a complex capable of specifically binding to mammalian serum albumin.

9. The use as claimed in claim 8, characterized in that said supporting peptide is a peptide fragment derived from streptococcal G protein.

10. The use as claimed in either of claims 8 and 9, characterized in that said complex is prepared by genetic recombination.

11. The use as claimed in one of claims 7 to 10, characterized in that said antigen, hapten or complex is covalently coupled with at least one of the compounds contained in the membrane fraction.

12. The use as claimed in claim 11, characterized in that the covalent coupling is a coupling carried out by chemical synthesis.

13. The use as claimed in claim 12, characterized in that there are introduced one or more linking elements into at least one of the compounds contained in the membrane fraction and/or in said antigen, hapten or complex to facilitate the chemical coupling.

14. The use as claimed in claim 13, characterized in that said linking element introduced is an amino acid.

15. The use as claimed in claim 11, characterized in that the coupling between said antigen, hapten or complex and at least one of the compounds contained in the membrane fraction is carried out by genetic recombination when said antigen, hapten or complex and said membrane compound are of a peptide nature.

16. The use as claimed in one of claims 1 to 15, characterized in that the pharmaceutical composition comprises, in addition, an agent which makes it possible to carry said membrane fraction associated with said antigen, hapten or complex in a form which makes it possible to enhance its stability and/or its immunogenecity.

17. The use as claimed in claim 16, characterized in that said agent is an oil-in-water or water-in-oil type emulsion.

18. The use as claimed in claim 16, characterized in that said agent is a particle of the liposome, microsphere or nanosphere type or any type of structure allowing the encapsulation and the presentation in particulate form of said membrane fraction associated with said antigen, hapten or complex.

19. The use as claimed in claim 16, characterized in that said agent is chosen from aluminum salts, calcium salts, compounds of plant origin such as Quil A or saponin, or compounds of bacterial origin such as cholera, pertussis or tetanus toxoid or thermolabile E. coli toxin.

20. The use as claimed in claims 1 to 19, characterized in that the pharmaceutical composition comprises, in addition, an agent which makes it possible to regulate the immune response induced by said membrane fraction associated with said antigen, hapten or complex.

21. The use as claimed in claim 20, characterized in that said regulatory agent is chosen from cytokines, growth factors, hormones or cellular components such as nucleic acids, a protein of the family of heat shock proteins or ribosomes.

22. The use as claimed in one of claims 1 to 21, for the preparation of a pharmaceutical composition intended for the prevention or treatment of infectious diseases or cancers.

23. The use as claimed in claim 22, characterized in that the infectious disease is of viral, bacterial, fungal or parasitic origin.

24. The use as claimed in claim 23, for the preparation of a pharmaceutical composition intended for the prevention or treatment of paramyxovirus infections.

25. The use as claimed in claim 24, characterized in that the paramyxovirus is a respiratory syncytial virus.

26. The use as claimed in claim 25, characterized in that said antigen associated with the membrane fraction comprises the peptide G2Na having the sequence SEQ ID No. 4 or one of its homologs whose sequence exhibits a degree of identity of at least 80% with the sequence SEQ ID No. 4.

27. The use as claimed in claim 26, characterized in that said peptide G2Na or one of its homologs is covalently coupled with a C-terminal fragment (BB) of the streptococcal G protein to form a complex capable of binding to mammalian serum albumin.

28. The use as claimed in claim 24, characterized in that the paramyxovirus is a parainfluenzae virus.

29. A pharmaceutical composition, characterized in that it comprises a membrane fraction prepared by the method as defined in either of claims 3 and 4, and an antigen or hapten associated with said membrane fraction.

30. The pharmaceutical composition as claimed in claim 29, characterized in that said antigen is chosen from paramyxovirus peptide fragments.

31. The pharmaceutical composition as claimed in claim 30, characterized in that the paramyxovirus is a respiratory syncytial virus or a parainfluenzae virus.

32. The pharmaceutical composition as claimed in claim 31, characterized in that said antigen associated with the membrane fraction comprises the peptide G2Na having the sequence SEQ ID No. 4 of the respiratory syncytial virus or a peptide whose sequence exhibits a degree of identity of at least 80% with the sequence SEQ ID No. 4.

33. The pharmaceutical composition as claimed in claim 32, characterized in that said peptide G2Na or one of its homologs is covalently coupled with a C-terminal fragment (BB) of the streptococcal G protein to form a complex capable of binding to mammalian serum albumin.